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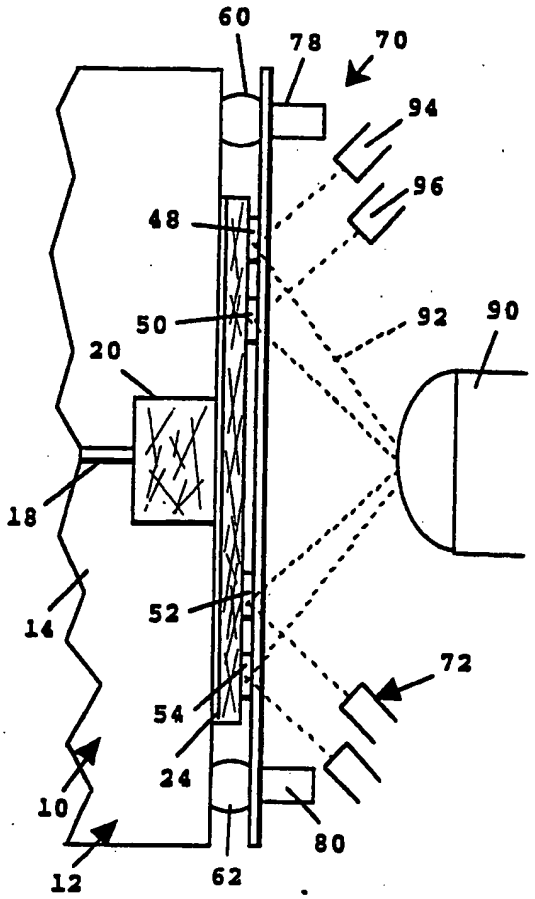
(54) Title: ANALYTE ASSAY DEVICE AND APPARATUS

(57) Abstract

Apparatus (70) and device (10) for use in determining the concentration of a selected analyte in a body-fluid sample. The device includes a sample dispenser (12) designed to distribute a small-volume blood sample to multiple transfer sites (30, 34, 36, 38, 40), by capillary flow of the blood sample through sieving and distributing matrices (22, 26) which separate blood cells from serum as the sample fluid migrates toward the transfer sites. A test plate (44) in the device carries multiple absorbent test pads (48, 50, 52, 54), each containing reagent components for use in detection of a selected analyte. The test plate is mounted on the dispenser for movement toward and away from a transfer position at which the exposed surface regions of the pads are in contact with associated sample-transfer sites, for simultaneous transfer of sample fluid from such sites to the pads in the support. The apparatus is designed for use in transferring a uniform, quantifiable amount of sample fluid to each of the pads in the device.



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ANALYTE ASSAY DEVICE AND APPARATUS

1. Field of the Invention

The present invention relates to an apparatus and device for use in determining the concentration of an analyte, and preferably, of multiple analytes in a small-volume sample, such as a blood sample.

2. Background of The Invention

Assays for detecting the presence and levels of a variety of analytes in body fluid samples are known. Such assays are often designed for simplicity of use so that they can be reliably conducted in a doctor's office or other clinical setting where personnel may have little training in clinical assay procedures or in interpreting assay results. Typically, such assays involve a one-step assay procedure, or employ automated or semi-automated procedures, with the assay reading being determined from a reaction end-point.

One type of diagnostic assay format which is generally amenable to simple, one-step assays involves an absorptive-pad device containing a pad or matrix designed to absorb a sample volume. The pad contains an analyte-dependent chemical reaction which can be detected on the pad surface. Examples of absorptive-pad assay devices and methods include U.S. Patent Nos. 3,983,005, 4,069,017, 4,144,306 and 4,447,575.

A number of factors have heretofore limited the accuracy of assays which are based on a conventional reaction matrix or pad format. One limitation is the variable amount of detectable reaction product formed in the presence of a given amount of analyte, due to variations in the stability of the reagents in the pad, variations in temperature and other reaction conditions, as well as the presence of variable amounts of interfering compounds in the analyte-containing sample.

These sources of variability can be largely eliminated in a three-pad self-corrected assay system and method in which analyte concentration is determined from a standard curve, and corrected for intereference based on the measured signal product readings from the three pads. Such assay system is described in co-owned U.S. patent application for "Self-Corrected Assay Method and System," Serial No. 238,775 filed August 30, 1988.

Increasingly, it is desirable in analyte testing to test for a battery of different analytes, and particularly when testing serum lipid levels, such as cholesterol, cholesterol subfractions, and triglycerides. Further, blood testing procedures are greatly simplified if the blood sample used for testing is a drop of blood, rather than a larger blood sample obtained by needle and syringe (which requires trained medical personnel) and which typically involves additional handling to separate blood serum or plasma from blood cells. Heretofore, however, multianalyte testing on small volumes has been limited by problems of obtaining accurate, uniform small sample volumes on multiple assay pads.

In summary, what is desired, but has heretofore not been attained, is a simple dry-pad sample assay device which (a) is designed for multiple analyte assays, (b) may employ a single drop of sample fluid, including whole blood, as the test material, and (c) provides an accurate, relatively

uniform sample volume delivery to multiple absorption assay pads.

3. Summary of the Invention

It is one general object of the invention to provide an absorption-pad assay device for performing multiple analyte tests on a small-volume blood sample.

It is a more specific object of the invention to provide such a device for use in testing multiple blood analytes, using a single drop of blood of 50 µl or less as the test sample.

It is another specific object of the invention to provide such a device and apparatus in which substantially the same amount of sample material is taken up into multiple absorbent assay pads.

The assay device of the invention includes a sample dispenser having a support, and a sieving matrix on the support which functions as a chromatography medium to retard blood cells of a blood sample being drawn through the matrix by capillary flow. Downstream of the sieving matrix is a second capillary-flow matrix which distributes the sample to multiple sample-transfer sites. The total absorption volume of the two matrices is preferably less than 50 µl. Residual blood cells remaining in the blood fluid sample after passage through the first matrix are removed upstream of the sample-transfer sites by (i) migration through the second matrix and/or (ii) a microporous membrane interposed between the two matrices.

A test plate in the device carries a plurality of wettable, absorbent assay pads, each having an exposed surface region and containing reagents for reacting with a selected analyte, when sample fluid is transfered to the pad, for analyte detection. The plate is mounted on the sample dispenser for movement toward and away from a sample-transfer position at which the surface regions of the pads are in contact with associated sample-transfer sites, for simul-

taneous transfer of cell-free sample fluid from the transfer sites to the assay pads.

In one embodiment, for use in assaying serum cholesterol and lipoproteins, the test plate includes first, second, and third pads, each of which includes, as assay reagents, a peroxidase and a dye which is converted in the presence of H_2O_2 to a detectable signal reaction product. The reagents in the pads further include:

(i) in the first pad, a known amount of a reference compound which is not present in the blood sample, and a reference-compound oxidase effective to generate H_2O_2 by reaction with the reference compound,

(ii) in the second pad, cholesterol esterase and cholesterol oxidase enzymes, and

(iii) in the third pad, triglyceride hydrolase, L-glycerol kinase, and L-glycerol-3-phosphate oxidase.

The reference compound is preferably a D-amino acid, and the reference-compound oxidase is D-amino acid oxidase.

In another aspect, the invention includes a diagnostic apparatus for use in determining the concentration of multiple analytes in a small-volume body-fluid sample. The apparatus includes an assay device of the type described above. More specifically, the device includes a dispenser having (i) a support, and (ii) distributing structure for distributing sample liquid applied to the support to a plurality of sample-transfer sites in the dispenser. A test plate in the device carries a plurality of wettable, absorbent pads, each having an exposed surface region and reagent components for detection of a selected analyte, when sample fluid is transferred to the pad. The test plate is mounted on the dispenser for movement toward and away from a sample-transfer position at which the surface regions of the pads are in contact with associated sample-transfer sites, for simultaneous transfer of sample fluid from such sites to the pads in the support.

A diagnostic instrument in the apparatus includes a holder for releasably supporting the assay device, and a shifting mechanism associated with the holder for shifting the dispenser and test plate in the device relatively toward and away from a sample-transfer position. A control element in the instrument functions to control the time at which the test plate is maintained in its sample-transfer position, to control the volume of fluid transferred to the pads.

In one embodiment, the control element is designed to maintain the sample-transfer contact position for a preselected time period. In another embodiment, the control element includes a detector for monitoring the extent to which said expanse has been wetted, and an operative connection between the detector and shifting mechanism, for discontinuing delivery of sample to the pads when the pads have been wetted to a given extent.

In a more general aspect, the invention includes a diagnostic instrument for use with an assay device composed of a sample dispenser designed to distribute a sample applied to the dispenser to a sample-transfer site, and a test plate having a wettable, absorbent pad, and mounted on the dispenser for movement toward and away from a sample-transfer position.

The instrument includes a shifting mechanism for moving the test plate to its sample transfer position, a detector for monitoring the extent to which the pad has been wetted, and a control element operatively connecting the shifting mechanism and detector, for discontinuing delivery of said sample when the pad has been wetted to a given extent, as monitored by the detector.

Further objects, features and advantages of the present invention will become apparent from the following detailed description of the invention, when read in conjunction with the accompanying drawings.

Brief Description of The Drawings

Figure 1 is a plan view of a multiple-analyte assay device constructed according to one aspect of the invention;

Figure 2 is a sectional view taken along line 2-2 of Figure 1;

Figure 3 is a sectional view taken along line 3-3 in Figure 1;

Figure 4 is a plan view similar to Figure 1, but showing the assay device in a sample-transfer position;

Figure 5 is an enlarged side view of a reaction pad in the assay device, and an optical beam and sensor used for monitoring the change in reflectance at the back surface of the pad due to sample fluid flow into and through the reaction pad;

Figure 6 plots the change in reflectance R as a function of time during the period of sample fluid delivery into and through a reaction pad in the assay device of Figure 1;

Figures 7A and 7B are plan views of the assay device of Figure 1 in sample-transfer and non-transfer conditions, respectively, shown with part of the diagnostic instrument for determining analyte concentration according to the invention;

Figure 8 is a schematic diagram of components of one embodiment of the apparatus of the invention; and

Figure 9 is a partly schematic view of portions of an apparatus constructed according to further embodiment of the invention in which sample fluid is delivered to a reaction pad by spraying.

Detailed Description of the Invention

A. Assay Device

Figures 1-4 illustrate a sample delivery device 10 constructed according to the invention. A sample dispenser 12 in the device generally includes a support 14 which defines a well 16 dimensioned and sized to receive a quantity of a blood sample, and typically between about 25-50 µl

of blood. A capillary conduit 18 formed in the plate is provided at the base of the well and communicates with notched region 20 formed in the upper edge of the support. The construction of the well, tube and notched region in the support can be appreciated from Figures 1 and 3. The support is preferably a thin plastic plate or the like, with the well, tube and notched region formed by standard molding or machining methods.

A sieving matrix 22 carried in region 20 functions to partially remove blood cells (including blood cells and other large particulate material in the blood sample) as the sample migrates through the matrix in a bottom-to-top direction in the figures. Matrix 22 is preferably formed of a fibrous matrix filter material designed to draw aqueous fluid by surface wetting, and to retard the movement of blood cells as the blood sample is drawn through the matrix. That is, the matrix serves as a chromatographic medium for separating cell-size particles from soluble serum components on the basis of different migration rates through the medium.

A variety of fibrous materials, such as are used in fibrous-mat filters, including cellulose, cellulose acetate, and glass fibrous matrices, are suitable materials for matrix. The fibers may be crosslinked, if desired, by chemical crosslinking, heat fusion, or the like. Also suitable are porous substrates, such as sintered glass, fused polymer beads, and the like whose wettability and dimension of interstices are such as to promote movement of an aqueous medium into the matrix by surface wetting. One exemplary filter is a glass fiber filter having a packing density of about 0.5 gm/cm^3, side dimensions of between about 3 mm, and a thickness of about 125 μ. The pad is dimensioned to absorb a defined volume of sample fluid, typically about 3-25 μl, and preferably between about 15-25 μl.

The upper surface of matrix 22 is covered by a microporous membrane 24. This membrane is designed to filter out

blood cells and other particulate matter present in the fluid sample. Where the device is used for assaying total cholesterol or other lipid components which may be associated with large lipoprotein bodies in the blood, e.g., high density liposproteins (HDLs), low-density lipoproteins (LDLs), and very-low density lipoproteins (VLDLs), the membrane pore sizes are selected to filter out blood cells, but allow allow passage of these lipid bodies. One preferred membrane is a polycarbonate membrane available from Nuclepore (Livermore, CA) and having a 1 micron pore size. The membrane is also referred to herein as filter means.

Membrane 24, in turn, is covered by an absorbent strip 26 which is attached to and extends along an interior portion of the plate's upper edge. Strip 26 serves to distribute sample fluid from a central region 28 of the strip, which is contact with matrix 22 through membrane 26, to opposite end regions 30, 32 of the strip, and more particularly, to multiple sample-transfer sites at opposite end regions of the strip, such as sites 34, 36 in end region 30, and sites 38, 40 in end region 32 (Figure 1). The strip is also referred to herein as a distributing matrix or distributing means for distributing sample from the sieving matrix to multiple sample-transfer sites in the distributing matrix.

The strip preferably has a lower fiber density than the matrix, giving greater fluid flow rate through the pad than through the strip. Strip 26 is formed of a fibrous material, such as employed in the sieving matrix, which is capable of drawing fluid through the strip by capillary flow. One exemplary strip material is a glass fiber filter having a packing density of about 0.2 gm/cm^3, a thickness of about 125 μ, and a length of about 3 cm. As seen in Figure 3, the sieving matrix, membrane, and strip and membrane all have about the same width dimension as plate 16, typically between 1-5 mm.

In operation, a blood sample, typically 25-40 µl, is introduced into well 16, from which it is drawn by tube 18 into matrix 22. As the sample is drawn through the matrix by capillary flow, cellular components in the blood are retarded and the leading edge of the blood sample becomes progressively depleted of cell components. The reduced concentration of the blood cells of the sample material which reaches membrane 24 reduces the tendency of the membrane to clog as sample material is drawn though the membrane by capillary flow into strip 26. After passage through membrane 24, the sample is essentially a cell-free plasma fluid which is then drawn toward the sample-transfer regions at the opposite end regions of the strip.

In one alternate embodiment, the filter means for completely removing blood cells from sample at the sample-transfer sites is provided by the distributing matrix or strip itself. Here the sieving and distributing matrices act as a continuous chromatography medium which is effective to completely separate the faster-migrating serum from the slower-migrating blood cell, when the sample first reaches the sample-transfer sites in the dispenser.

In still another embodiment, the filter means is a microporous membrane like membrane 24, but placed over the outer surface of the distributing matrix. Here the sample transfer sites are on the outermost surface of the membrane, corresponding to the opposite end regions of the underlying distributing matrix. As in the above-described embodiments, the filter means here acts to substantially completely remove blood cells and similar-size or larger particulate material in the blood sample as the sample migrates to the sample-distribution sites.

With continued reference to Figures 1-4, device 10 includes a test plate 44 composed of an elongate strip 46, and multiple wettable, absorbent test pads 48, 50, 52, and 54 carried on the lower surface of the support, at positions directly above the four corresponding sample-transfer sites

34, 36, 38, and 40 in the dispenser, respectively. That is, the four pads are positioned on the test plate for contact with the corresponding transfer sites in the dispenser, when the support is moved to a sample-delivery position, described below with respect to Figure 4. The strip is transparent or has transparent windows which allow the pads to be viewed through the strip.

In the present embodiment, the device includes four sample-transfer sites and four associated pads. More generally, the device includes at least two and up to six or more pads and sample-transfer sites.

The pads in the test plate are attached to the support by a transparent or translucent adhesive material 56 (Figure 5), or by sonic welding or other suitable bonding method. Each pad contains analyte-dependent reagents effective to produce an analyte-dependent change in the pad which can be detected optically, either visually or by a detector, in a known manner. The nature of the reagents for exemplary analyte assays is given in Section B. Preferably, the reaction pads are porous, fused polymer or microporous polymer membranes having a thickness, after complete penetration of the fluid, of about 100-150 μ and side dimensions of about 3 mm. Preferred membranes are polysulfone, polypropylene, nylon, nitrocellulose, Teflon™, or polyvinylchloride microporous membranes having a pore size preferably between about 0.1-10 μ, and preferably 0.3 to 1 μ. Polysulfone membranes are advantageous in that the membrane is relatively opaque when it absorbs a clear fluid sample. The absorption volume of each pad is preferably between about 0.5-2 μl.

The test plate is mounted on the dispenser by a pair of resilient members, such as elastomeric blocks 60, 62. The blocks act to bias the pads toward a non-transfer position at which the pads are spaced apart from the dispenser's sample-transfer surface, with a spacing typically of between about 0.5 to 1.0 mm.

In operation, when a serum sample reaches the sample-transfer sites in the dispenser, the test plate is moved toward its sample-transfer position (Figure 4), at which the exposed, contronting surfaces of the pads are in contact with the corresponding transfer sites. At this position, sample fluid in the dispenser is drawn into the pads by capillary flow with fluid movement occurring in a direction normal to the pad surfaces. The plate is held at this position until a desired degree of wetting of the pads is achieved. The degree of wetting may be followed, for example, by the change in reflectance of the pads, as viewed from the top, since the reflectance of the pads decreases as the pads are wetted. Alternatively, the time required for optimal pad wetting, at a selected contact pressure and pad material and thickness, can be calibrated. The pads are then wet under the selected pressure conditions for the calibrated time. Typically, the contact time for pad filling is set at 2-3 seconds.

B. Multiple Analyte Assays

The assay device of the invention is designed particularly for determination of blood analytes such as lipids, in which a battery of different lipid analytes are part of an overall diagnostic test. In one preferred example, described below, the device is designed for assay of total serum cholesterol and serum triglycerides, and optionally for determination of HDL cholesterol subfraction, such as free and esterified serum cholesterol. In a more general preferred configuration, the device is designed for self-corrected analyte determinations of multiple analytes, as described in co-pending U.S. patent application for "Self-Corrected Assay Device and Method," Serial No. 369,326, filed August 23, 1989.

In this more general configuration, the test pads each contain common-pathway reagent components for converting H_2O_2 to a distinctly colored signal reaction product. The

components include peroxidase, and a dye (meaning a single dye or coupled dye system) which can be converted by the peroxidase, in the presence of H_2O_2, to a distinctively colored signal reaction product. The peroxidase enzyme is a hydrogen-peroxide oxidoreductase, such as horseradish peroxidase, myeloperoxidase, and lactoperoxidase, which catalyses the reaction:

Donor + H_2O_2 → oxidized donor + $2H_2O_2$.

The specificity of the enzyme for the donor is generally low, and a number of phenols, aminophenols, diamines, and indolephenols are active. In the present invention, the donor is selected among a variety of known compounds or pairs of compounds which undergo reaction to a detectable, typically chromogenic reaction product as a result of peroxidase-catalysed oxidation.

Exemplary donor compounds include O-pheylenediamine, amidopyrine, and naphthalene-2,3-dicarboxaldehyde. Typically formation of a colored reaction product involves dimer formation.

In addition to components, the first test pad of the common pathway, which is used as a self-correcting standard, the reagents in the pad also includes a known amount of reference compound which is not present in the blood sample, and an oxidase enzyme effective to generate H_2O_2 by reaction with the reference compound. A preferred reference compound is a D-amino acid, and a preferred oxidase, D-amino acid oxidase. The reference compound and oxidase enzymes are preferably contained in separate layers of the test pad, as described in the above-referenced patent application, Serial No. 369,326.

When sample fluid is introduced into this first pad, the reference compound is brought into solution and into contact with the oxidase enzyme, with the generation of H_2O_2 and H_2O_2-dependent generation of colored reaction product. The intensity of the colored reaction product will depend (i) the amount of known reference compound in the pad (which

is known), (ii) the condition of the enzyme reagents in the pad, including the oxidase enzyme, (iii) the reaction conditions, such as ambient temperature, and (iv) the inhibitory effect of components in the blood sample fluid. It will be appreciated that factors (ii-iv) are applicable to the the common-pathway components for color generation from H_2O_2 present in each of the test pads, and thus the reference pad provides a self-correcting standard for each of the other test pads.

The remaining test pads contain reagent components effective to generate H_2O_2 by reaction with selected analytes, including an oxidase enzyme which is specific for the selected substrate. Table I shows several exemplary analytes for which suitable analyte-specific oxidases exist. As seen, the analytes may themselves be the substrate of the analyte-specific enzyme, as in the case of glucose, uric acid, amino acid oxidase, and free (non-esterified) cholesterol. Here the analyte-specific oxidase reagents may include only the oxidase enzyme.

Alternatively, the analyte may be first converted by primary analyte-specific enzyme(s) to produce the substrate recognized by the oxidase enzyme. Here the analyte-specific oxidase reagents include both the oxidase and additional enzyme for converting the analyte to the oxidase substrate.

In the case of esterified cholesterol, for example, the analyte-specific oxidase reagents include cholesterol esterase, for converting cholesterol in esterified form to free cholesterol, and cholesterol oxidase, which produces cholestenone and H_2O_2 in the presence of oxygen.

The analyte-specific oxidase reagents for determination of serum triglyceride include lipase, which hydrolyses triglyceride to glycerol and free fatty acids; glycerol kinase, which converts glycerol to glycerol-3-phosphate in the presence of ATP; an ATP-generating system; and glycerol-3-phosphate oxidase, which reacts with glycerol-3-phosphate to produce dihydroxyacetone-phosphate plus H_2O_2.

The analyte-specific oxidase reagents for determination of creatinine include creatinine amidohydrolase, which converts creatinine to urea and sarcosine, and sarcosine oxidase, which converts sarcosine to glycine and formaldehyde, with production of H_2O_2.

TABLE I

Analyte	Substrate	Oxidase
glucose	glucose	glucose oxidase
uric acid	uric acid	uricase
amino acid	amino acid	amino acid oxidase
free cholesterol	cholesterol	cholesterol oxidase
esterified cholesterol	cholesterol	cholesterol oxidase
triglyceride	L-glycerol-3-phosphate	L-glycerol-3-phosphate oxidase
creatinine	sarcosine	sarcosine oxidase

One exemplary three-pad device, designed for determination of total serum cholesterol and triglyceride, contains in each pad, in addition to the above common-pathway peroxidase and dye components:

(i) in the first pad, a known amount of a reference compound which is not present in the blood sample, and a reference-compound oxidase effective to generate H_2O_2 by reaction with the reference compound,

(ii) in the second pad, cholesterol esterase and cholesterol oxidase enzymes, for generating H_2O_2 from free and esterified serum cholesterol and

(iii) in the third pad, triglyceride hydrolase, L-glycerol kinase, and L-glycerol-3-phosphate oxidase, for generating H_2O_2 from triglyceride.

A fourth test pad in the above device may be designed for one of a variety of tests. In one embodiment, the

fourth test pad contains the same reagent components as the first pad, but with a different known amount of reference compound. This configuration is used in generating a two-point standard correction curve, as described in the above-referenced patent application, Serial No. 396,326.

In a second embodiment, the fourth reaction contains the same reagent components as the second pad, except that cholesterol esterase is absent. This pad thus generates H_2O_2 from free cholesterol, but not esterified cholesterol, and can be used, in conjunction with the total cholesterol test pad reading, to calculate total esterified cholesterol.

In a third embodiment, the fourth pad contains reagent enzymes, such as glucose oxidase, for assay of other serum components, such as glucose. It will be appreciated that the test device may contain more than three or four test pads, and thus incorporate all of the above variants and/or other analyte tests.

From the foregoing, it can be appreciated how various objects and features of the assay device are met. First, the device provides accurate analyte determination of multiple blood analytes present from a single drop of whole blood, typically less than 40-50 µl volume. Secondly, the device acts to separate blood cells from blood fluid, to eliminate color and other cell-related interference with the assay. Thirdly, the volume of sample which is transferred to the test pads can be controlled and quantitated, to improve the accuracy of assay determinations (expressed as analyte concentrations).

Several features of the device are important for achieving the above results. The dispenser construction in the device allows for a small blood-sample volume to be distributed to multiple dispenser sites, with removal of blood cells as the sample migrates to the sites. The test plate allows sample fluid to be transferred at a selected transfer time when the volume of sample at each sample transfer site is most nearly equal. That is, sample-

transfer can be timed to correspond to the time point when all of the sample transfer sites in the dispenser first fill. Further, where the cell filtering in the device is accomplished entirely by chromatographic separation in the sieving and distributing matrices, sample transfer can be carried out after all of the sample-transfer sites are filled, but before migration of any blood cells into the sites occurs.

The test plate construction, and its interaction with the dispenser, permits timed or controlled volume transfer to the test pads. Since the volume transfer occurs across the entire outer surface of the pad, i.e., in a direction normal to the surface of the pads, reagent components in the pad are dissolved uniformly throughout the pad in the sample fluid.

In a preferred embodiment of the device, the test pads have common end-reaction reagents which convert an intermediate reaction product, H_2O_2, to a detectable product, and at least one of the pads includes a reference compound which utilizes the common reagents. This configuration allows for (i) determination of analyte concentrations based on standard curve and (2) self-consistent correction for errors which may result from loss of activity of the common pathway reaction components, inhibitory effects of the sample on the common-pathway reaction components, and temperature and other reaction-condition effects.

C. Assay Apparatus

In another aspect, the invention includes a diagnostic apparatus for use in determining the concentration of multiple analytes in a small-volume body-fluid sample. The apparatus, which is indicated generally at 70 in Figures 5, 7 and 8, generally includes an assay device 10 such as described in Sections A and B above, and a diagnostic instrument 72 which is designed to (i) performed a timed sample transfer frin the dispenser to the pads in the assay device

and (ii) measure analyte concentration levels from chemical changes occurring on the reaction pads.

Turning now to Figure 8, the diagnostic instrument of the invention includes a sample holder 74 which receives the assay device releasably. The holder provides a plunger 76 designed to engage and support the dispenser, and a pair of arms 78, 80 which are designed to engage and support opposite sides of the test plate in device 10, as shown. The plunger is connected to the piston 82 of a solenoid 84, for movement therewith between a delivery position, at which the device is placed in a sample-transfer position, and a non-transfer position at which the device is in its normal spaced-apart configuration shown in Figure 1.

As indicated above, the solenoid is operable to initiate sample delivery in the device, by movement to its delivery position, and to hold the device at the sample-delivery position (Figure 7A) for a controlled period of time, after which sample delivery is discontinued (Figure 7B) by solenoid movement to the non-transfer position. The solenoid, piston, and plunger are also referred to herein as shifting means for shifting the assay device toward and away from its sample-transfer position.

The instrument further includes a controller 86 for controlling the time during which the test plate is maintained at its sample-transfer position, to control the volume of fluid transferred to the pads in the test plate. The controler is also referred to herein as a control element or control means.

In one embodiment, the control means includes a timer which can be set to hold the solenoid in a sample-transfer condition for a preselected time period, typically 1-5 seconds, and preferably 2-3 seconds, depending on the contact pressure, pad size, and nature of the pad material. The preselected time can be determined, for example, by calibrating the extent of pad filling at increasing time intervals, until a time which gives an optimal extent of

filling is first achieved. Once this time is set, the preselected time period can be applied to sample-transfer involving the same contact pressure, pad size, and pad material conditions.

In another embodiment, the control means includes detector means for monitoring the extent to which the pads have been filled, and means connecting the detector means to the shifting means, for discontinuing sample transfer (moving the test plate away from its sample-transfer position) when the pads in the test plate have been wetted to a given extent.

With reference particularly to Figures 5, 7, and 8, the detector means includes a light source 90 which produces directed light beams, such as beam 92, which are directed at an angle against each reaction pad, with assay device in holder 74. The detector means further includes, for each pad, a light detector, such as detectors 94, 96 associated with pads 48 and 50, respectively, seen in Figures 7A and 7B. The detectors are connected to the controller, as shown, for providing information about the detected reflectance from each pad to the controller.

As will be appreciated, during sample transfer, as liquid sample migrates into and through the pad in a direction normal to the pad surface, reflectivity of the surface of the pad will decrease, due to the greater translucency of the pad, which is typically white and relatively reflective in its dry condition. Figure 5 shows in enlarged scale the monitoring of a pad during sample transfer. Here beam 92 from the light source is directed through transparent plate 46 and the adhesive material 56 against the surface of pad 48. As the pad is wetted by the migration of liquid 98 through the pad, the intensity of the reflectance beam 100 measured by detector 94 decreases, until the pad becomes completely wetted.

Figure 6 shows typical plots of refectance R measured in three pads during sample transfer, where each pad has a

total absorption volume, for complete wetting of about 0.75 µl. The abscissa is a time scale, in hundredths of a seconds. Sample transfer is initiated at time t=0. All three pads show a sharp decrease in reflectance, over an approximately 2 second time period, after which reflectance plateaus, indicating complet wetting of the pad. As seen, each pad wetted completely at about the same rate.

In monitoring the change in reflectance from a pad surface, the amount of change can be determined and calculated so that when it reaches a predetermined value or predetermined differential value, based on previously generated data, it can be automatically determined that a desired amount of fluid sample has been delivered to the reagent pad. Then delivery can be discontinued, by retracting the solenoid to its non-transfer position.

The changes in reflectance measured by the detectors are monitored by the above controller which is operatively connected to te detectors through a multiplexer 104. The multiplexer operates conventionally to sample the signal outputs of the four detectors rapidly and sequentially, and input the signal outputs into the controller. As noted above, it has been determined that complete wetting of a 0.75µl volume pad requires about 2 seconds. Thus, it has been determined that reflectance measurements every 100 milliseconds will provide sufficient data (20 data points) and monitoring to terminate delivery close enough to the optimum 0.75 µl without substantially under-delivering or over-delivering specimen.

The controller is suitably programmed to respond to a given change of reflectance or absolute reflectance value, and is preferably set to operate a switch 106 in the control means for switching the solenoid to its delivery position for a period of time which is no more than that required to effect wetting of any pad to the selected extent as determined by the associated detector, and then to switch the

solenoid to its non-transfer position, to terminate sample transfer to the pads.

The control means desirably includes calculating means, such as a calculator 110, for calculating the rate of change of reflected light intensity received from the pad expanse at the optical detector, as sample is delivered to the pads. This calculation can be done conventionally, using, for example, a digital differentiator. Here switching to the non-delivery condition can be set to occur when the differential of the reflectance curve of any pad being monitored first reverses direction. The design of such a control means can be carried out readily according to well known solid-state design principles.

Preferably, the calculator is also designed to calculate the volume of liquid delivered to the pad, based on the change in reflected light intensity as monitored by the associated light detector. The volume calculation, in one embodiment, is made by determining the slope of the reflectance curve at the termination of sample-delivery to the pad, and correlating this slope with a predetermined table of values of slope vs. percent volume fill for a particular size pad.

In the embodiment shown, the detector means is also used to measure the change in reflectance in the associated pad due to the production of a colored reaction product in the pad, as analyte is utilized in forming the reaction product, after pad wetting occurs. As can be appreciated, when the light beam of the light source has a wavelength at or near the absorption maximum of the colored reaction product, the reflectance from the pad will decrease gradually with continued product of reaction product, until a new (second) reflectance-curve plateau is reached at the end point of the reaction. The total amount of analyte can then be calculated from the difference in reflectance at the first plateau (just after pad wetting) and at the second plateau (at the product end point). Alternatively, the

amount of analyte can be calculated from reaction kinetics, based on the rate of change of reflectance observed after pad wetting. Microprocessor designs for performing such calculations are known.

Based on the calculated volume of sample applied to a pad, and the amount of analyte contained in the volume, as determined by an analyte-dependent chemical reaction in the pad, the concentration of analyte in the sample can then be determined by calculator 110.

In the embodiment of Figures 1 to 4, the transfer of fluid sample results from physical contact of a wetted sample-distribution strip and the confronting surface of one or more reaction pads. As shown by Figure 9, sample transfer may alternatively be accomplished by a suitable sample spray transfer means by which fluid is sprayed onto one or more reaction pads in the apparatus. In such an apparatus, it is unnecessary to provide means, such as the resilient members in the sample-delivery device, and the shifting means in the diagnostic instrument, and other related components, for effecting transfer and delivery of the sample.

As such, the diagnostic apparatus of Figure 9, indicated generally at 112, comprises a multi-pad assay device, shown fragmentarily at 114, which is composed of a transparent test plate 116 and multiple reaction pads, such as pad 118. The attachment of the pads to the support by adhesive is as described above, and the wetting and formation of reaction product in the pad are monitored by suitable detectors means (not shown), as described above.

The assay device is supported in a suitable holder (not shown) in a diagnostic instrument 119 in the apparatus, which is like above-described instrument 72, except that the sample-transfer means in the instrument comprises a spray mechanism 120, or spray means, for spraying sample onto each of the reaction pads in the sample-transfer device. The spray mechanism includes a pump 122 which is under the

control of a microprocessor controller 124 via a suitable switch 126.

The pump causes suitable spray head or nozzles 128 in the mechanism to spray sample against the surface region of the pad or reaction pads, such as pad 118, until the detector system determines that the pad expanse has been sufficiently wetted, following which the pump is switched to its non-transfer condition.

From the foregoing, it will be appreciated how various objects and features of the apparatus are met. The apparatus provides an automated or semi-automated method for determining analyte concentrations in an applied sample. The method is easily performed, requiring only sample addition to a sample dispenser. In one embodiment, optimal sample-transfer time is determined by calibrating pad filling as a function of time, and an optimal sample-transfer time period is preselected for achieving uniform, selected-volume transfer to one or more pads.

In a second general embodiment, the extent of pad filling is monitored during sample transfer, and the amount of fluid in each of one or more pads can be quantitated, for accurate determination of analyte concentration, based on sample volume.

Although the invention has been described with reference to exemplary and preferred embodiments and configurations, it will be apparent to those skilled in the art that various changes and modification may be made without departing from the invention.

IT IS CLAIMED:

1. An assay device for use in assaying multiple analytes in a blood sample whose volume is no more than about 50 µl, comprising

a sample dispenser having (i) a support, (ii) a sieving matrix on said support effective to selectively retard blood cells when a blood sample is applied to one end of the pad and the sample migrates by capillary flow toward the opposite pad end, (iii) distributing means on said support in fluid contact with said opposite matrix end, for distributing sample liquid from the sieving matrix by capillary flow to a plurality of sample-transfer sites in the dispenser, and (iv) filter means interposed between said sieving matrix and said sample-transfer sites for removing substantially all of the blood cells from the blood sample before the sample reaches the sample-transfer sites,

a test plate carrying a plurality of wettable, absorbent pads, each having an exposed surface region and reagent means for detection of a selected analyte, when sample fluid is transfered to the pad, and

means mounting said plate on said dispenser for movement toward and away from a sample-transfer position at which the surface regions of the pads are in contact with associated sample-transfer sites, for simultaneous transfer of sample fluid from such sites to the pads in the support.

2. The assay device of claim 1, wherein said sieving matrix is a glass fiber matrix dimensioned to absorb a blood volume of less than about 25 µl.

3. The assay device of claim 2, wherein the distributing means is a glass fiber matrix strip dimensioned to absorb a sample volume of less than about 25 µl.

4. The assay device of claim 3, wherein said filter means includes a microporous membrane interposed between and in contact with said sieving matrix and strip.

5. The device of claim 2, wherein said distributing means is an elongate glass-fiber matrix strip, the strip communicates with the sieving matrix in the stri's center region, and said sample-transfer sites are at opposite end regions of the strip.

6. The device of claim 1, wherein said pads are microporous polymer substrate membranes each having a pore size of between 3-10 µ and an absorption volume of between about 0.5 to 2 µl.

7. The device of claim 6, wherein the pads are polysulfone membranes.

8. The device of claim 1, for use in assaying serum cholesterol and lipoproteins, wherein said test plate includes first, second, and third pads, the reagent means in each pad includes a peroxidase and a dye which is converted in the presence of H_2O_2 to a distinctly colored signal reaction product, and the reagent means in the pads further includes:

(i) in the first pad, a known amount of a reference compound which is not present in the blood sample, and a reference-compound oxidase effective to generate H_2O_2 by reaction with the reference compound,

(ii) in the second pad, cholesterol esterase and cholesterol oxidase enzymes, and

(iii) in the third pad, triglyceride hydrolase, L-glycerol kinase, and L-glycerol-3-phosphate oxidase.

9. The device of claim 7, wherein said reference compound is a D-amino acid, and the reference-compound oxidase is D-amino acid oxidase.

10. The device of claim 9, which further includes a fourth pad containing the same reagent means as the first pad, but a different known amount of the reference compound.

11. Diagnostic apparatus for use in determining the concentration of multiple analytes in a small-volume body-fluid sample, comprising

an assay device composed of (a) a sample dispenser having (i) a support, and (ii) distributing means on said support for distributing sample liquid applied to the support to a plurality of sample-transfer sites in the dispenser, (b) a test plate carrying a plurality of wettable, absorbent pads, each having an exposed surface region and reagent means for detection of a selected analyte, when sample fluid is transfered to the pad, and (c) means mounting the test plate on the dispenser for movement toward and away from a sample-transfer position at which the surface regions of the pads are in contact with associated sample-transfer sites, for simultaneous transfer of sample fluid from such sites to the pads in the support, and

a measuring instrument comprising (i) a holder for releasably supporting said dispenser (ii) shifting means operatively mounted on the holder for shifting said dispenser and test plate relatively toward and away from such sample-delivery position, (iii) means for controlling the time at which is test plate is maintained in its sample-transfer position, to control the volume of fluid transferred to the pads, and (iv) means for determining the

concentration of analyte present in the fluid sample transferred to each pad.

12. The apparatus of claim 11, wherein said controlling means includes timing means for holding the test plate in its sample-transfer position for a selected time.

13. The apparatus of claim 11, wherein said controlling means includes detector means for monitoring the extent to which said pads have been wetted, and means operatively connected to said shifting means and to said detector means for discontinuing delivery of said body fluid when said pad has been wetted to a given extent, as monitored by said detector means.

14. The apparatus of claim 11, for use in determining multiple analytes in a small-volume blood sample, wherein said dispenser device further includes a sieving matrix on said support effective to selectively retard blood cells when a blood sample is applied to one end of the matrix, and filter means interposed between the matrix and distributing means for removing substantially all blood cells before the cells reach the distributing means.

15. The apparatus of claim 14, for use in assaying serum cholesterol and lipoproteins, wherein said test plate includes first, second, and third pads, the reagent means in each pad includes a peroxidase and a dye which is converted in the presence of H_2O_2 to a distinctly colored signal reaction product, and the reagent means in the pads further includes:

(i) in the first pad, a known amount of a reference compound which is not present in the blood sample, and a reference-compound oxidase effective to generate H_2O_2 by reaction with the reference compound,

(ii) in the second pad, cholesterol esterase and cholesterol oxidase enzymes, and

(iii) in the third pad, triglyceride hydrolase, L-glycerol kinase, and L-glycerol-3-phosphate oxidase.

16. A diagnostic instrument for use with an assay device composed of (a) a sample dispenser designed to distribute a sample applied to the dispenser to a sample-transfer site, (b) a test plate having a wettable, absorbent pad, and (c) means mounting the test plate on the dispenser for movement toward and away from a sample-transfer position at which sample fluid is transfered from the sample-transfer site to the pad, said instrument comprising

means for shifting the test plate to its sample transfer ppsition,

detector means for monitoring the extent to which said expanse has been wetted, and

control means operatively connected to said shifting means and to said detector means for discontinuing delivery of said sample when said pad has been weeted to a given extent, as moniotred by said detector means.

17. The instrument of claim 16, wherein wherein said control means is operative to switch the shifting means to its delivery condition for a period of time which is no more than that required to effect complete wetting of the defined expanse of the pad, as monitored by said detector means.

18. The instrument of claim 16, for use in multi-analyte testing, wherein the dispenser includes multiple transfer sites, the test plate includes multiple pads which are designed to contact associated transfer sites, when the test plate is placed at its sample-transfer position, and the pads all fill at substantially the same rate, with the test plate in the sample-transfer position.

19. The instrument of claim 18, for use in assaying serum cholesterol and lipoproteins, wherein said test plate includes first, second, and third pads, the reagent means in each pad includes a peroxidase and a dye which is converted in the presence of H_2O_2 to a distinctly colored signal reaction product, and the reagent means in the pads further includes:

(i) in the first pad, a known amount of a reference compound which is not present in the blood sample, and a reference-compound oxidase effective to generate H_2O_2 by reaction with the reference compound,

(ii) in the second pad, cholesterol esterase and cholesterol oxidase enzymes, and

(iii) in the third pad, triglyceride hydrolase, L-glycerol kinase, and L-glycerol-3-phosphate oxidase.

20. The instrument of claim 19, wherein said reference compound is a D-amino acid, and the reference-compound oxidase is D-amino acid oxidase.

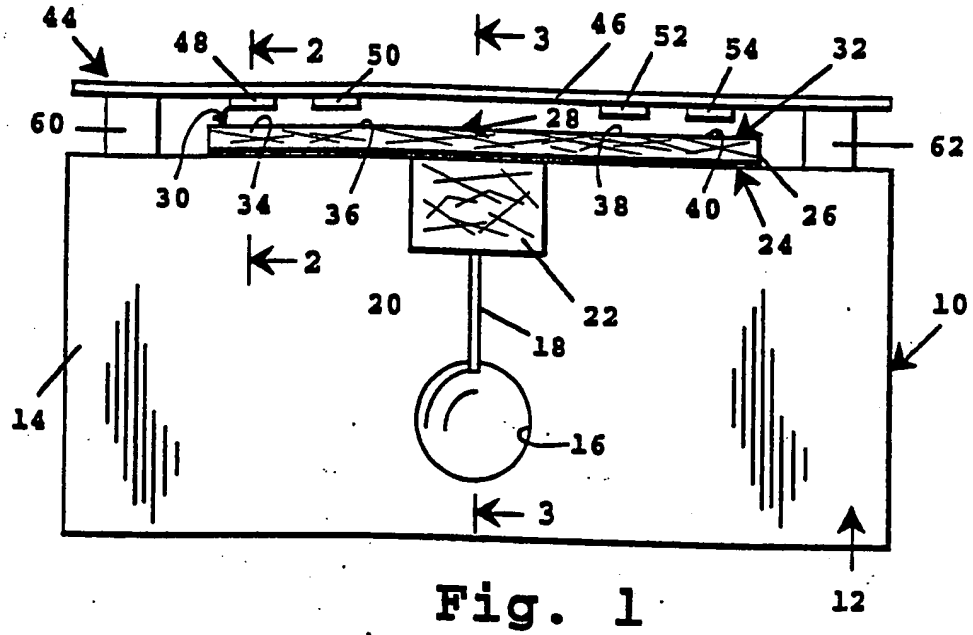

Fig. 1

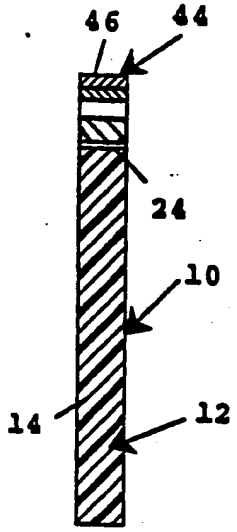

Fig. 2

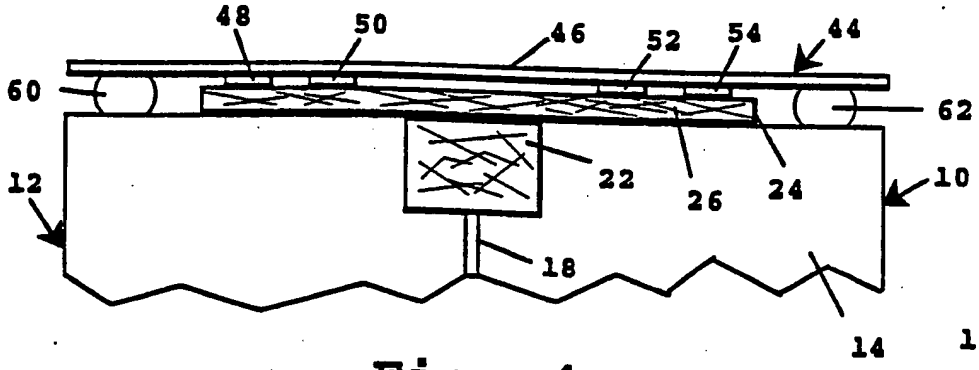

Fig. 4

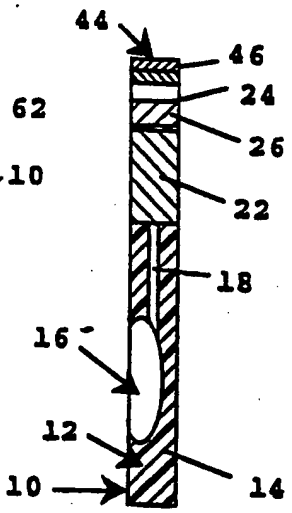

Fig. 3

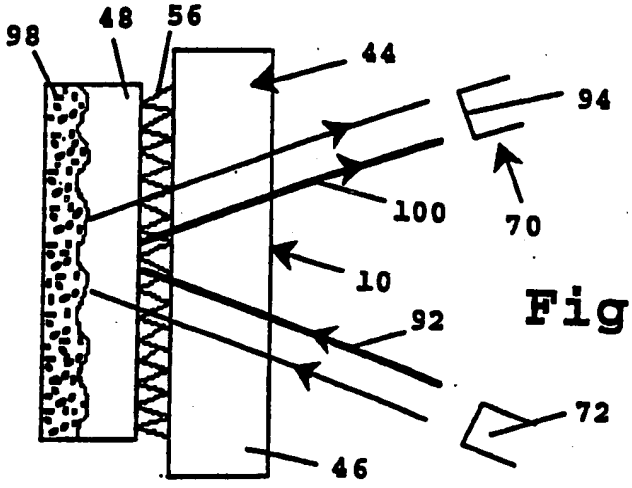

Fig. 5

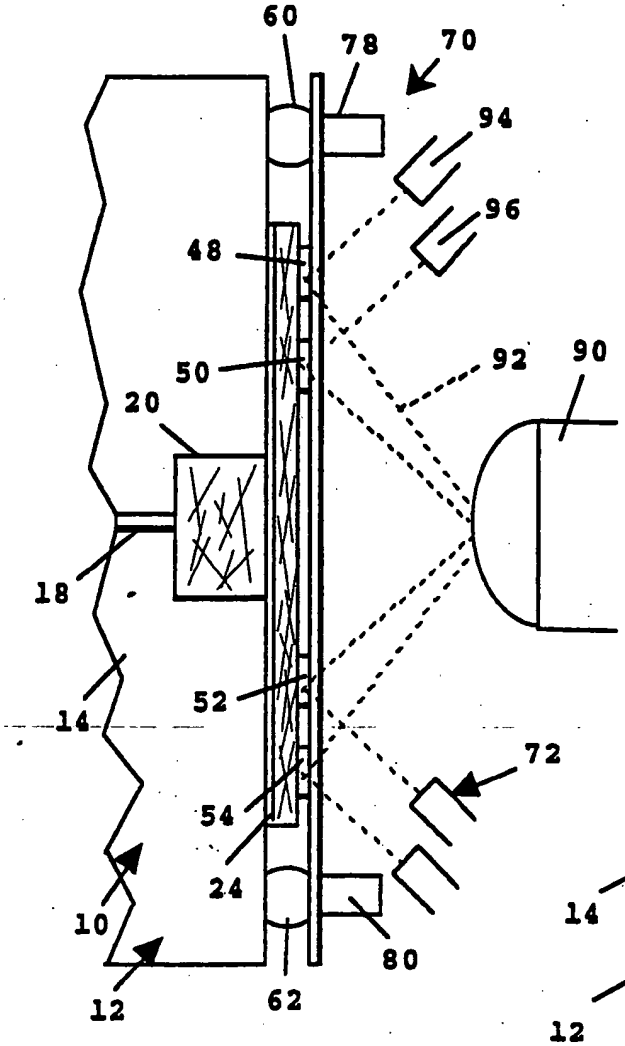
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Fig. 7A

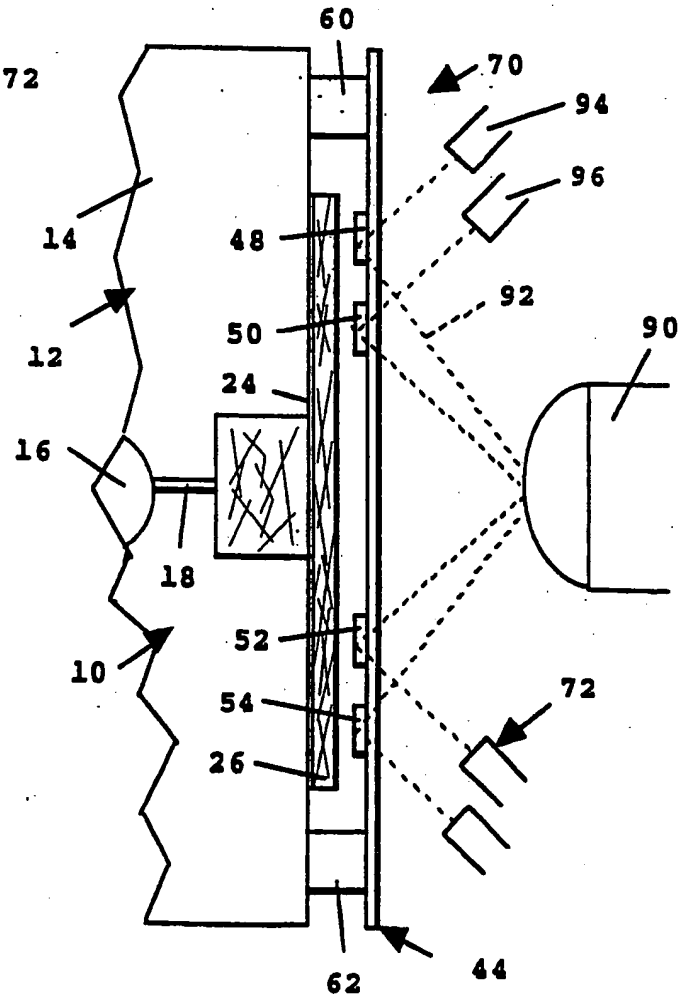
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Fig. 7B

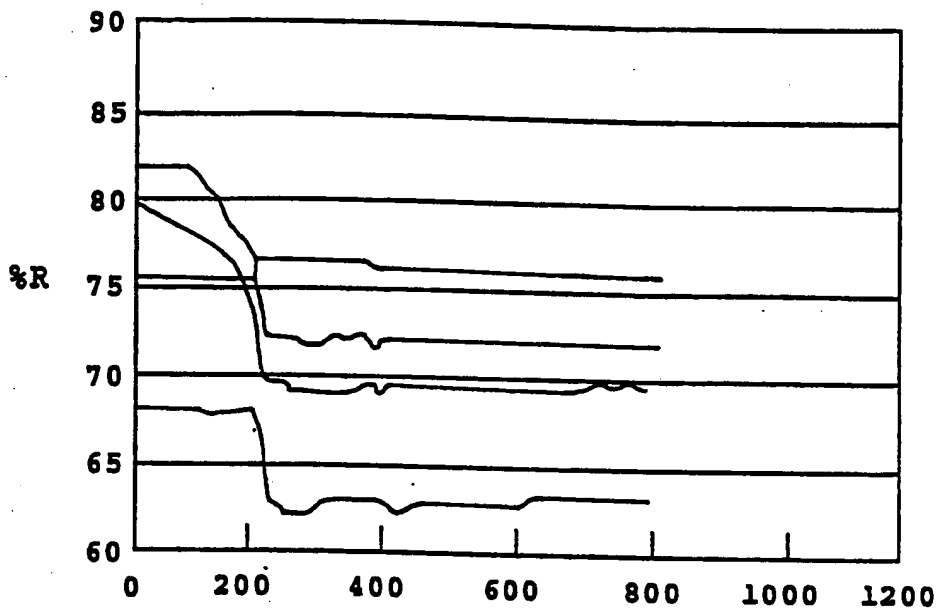

Fig. 6

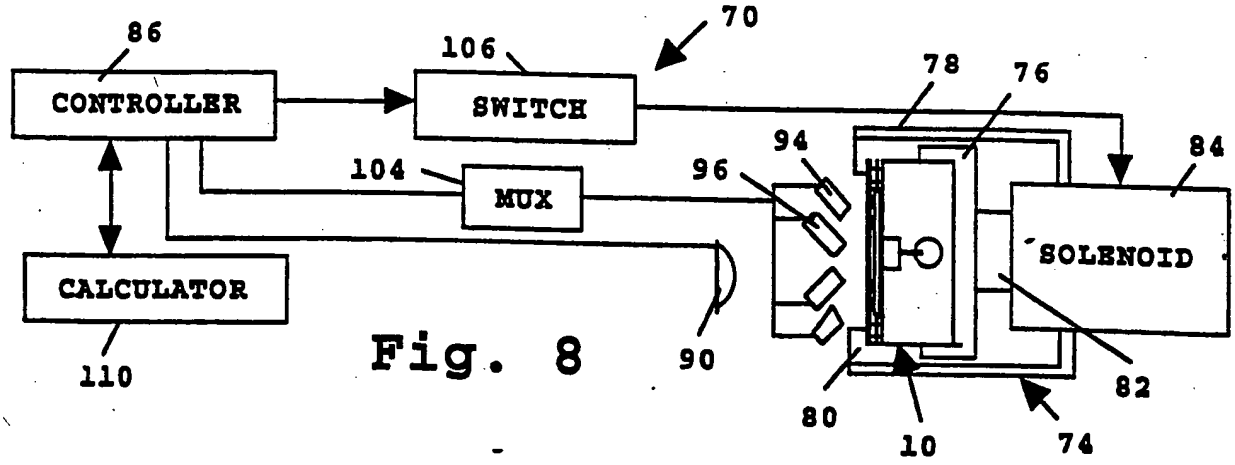

Fig. 8

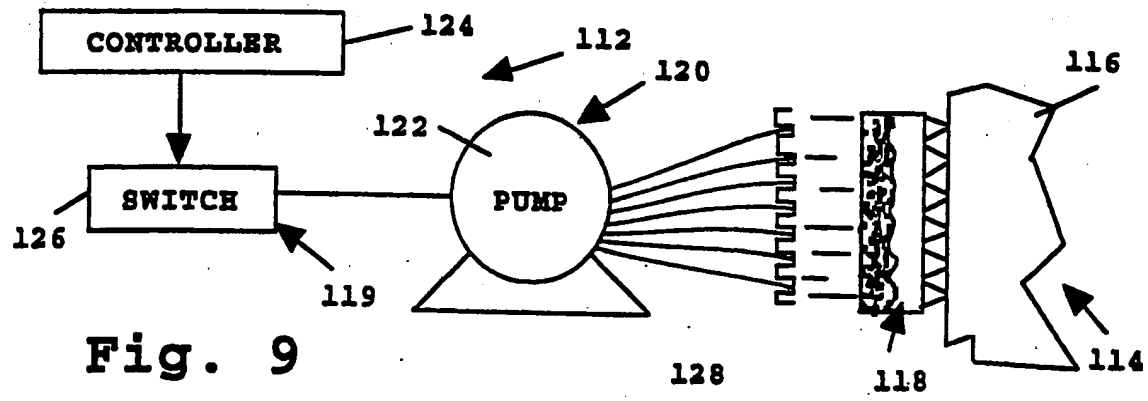

Fig. 9

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/01249

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: G 01 N 33/52, B 01 L 3/00, G 01 N 33/92, C 12 Q 1/60

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	B 01 L, G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 4549655 (J.G. FORSYTHE Jr. et al.) 29 October 1985 see abstract; figures 7,8 --	1
A	GB, A, 2090659 (INSTRUMENTATION LABORATORY INC.) 14 July 1982 see page 1, lines 25-68 --	1
A	EP, A, 0045476 (BOEHRINGER MANNHEIM GmbH) 10 February 1982 see page 15, line 10 - page 16, line 17 --	1
A	US, A, 4248829 (MASAO KITAJIMA et al.) 3 February 1981 see column 2, lines 17-57; column 4, lines 14-62; column 5, line 56 - column 6, line 26 -- ./.	1

[10] Special categories of cited documents:

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
7th June 1990	11. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	H Daniels A. DANIELS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO, A, 8300931 (EASTMAN KODAK CO.) 17 March 1983 see page 5, line 14 - page 6, line 3 --------	11,13,17

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9001249 SA 35392

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 03/07/90
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4549655	29-10-85	None		
GB-A- 2090659	14-07-82	AU-A-	7909581	08-07-82
		DE-A-	3151291	26-08-82
		FR-A-	2497577	09-07-82
		JP-A-	57132900	17-08-82
EP-A- 0045476	10-02-82	DE-A,C	3029579	18-02-82
		AU-B-	525394	04-11-82
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		US-A-	4477575	16-10-84
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		DE-A-	3278024	25-02-88
		EP-A,B	0087466	07-09-83

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